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# The studies on effect of of plant extract of *Vitex* negundo Linn against different bacterial species

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#### Abstract

The Methanol leaf extracts of of *Vitex negundo* were analyzed to study the antibacterial activity against three bactria i.e. *E. coli, Staphylococcus, Pseudomonas, Enterobacter, Salmonella typhimuri* using the disc diffusion method. The in *vitro* study was conducted by using 25 µl concetration. The five treatments and three replications were adopted durining the study. All the five bacteria *E. coli, Staphylococcus, Pseudomonas, Enterobacter, Salmonella typhimuri* were inhibited by leaf extract with zone of inhibition 23 mm, 22.1 mm, 19.6 mm, 18.5 mm and 17.8 mm respectively.

Keywords: Vitex negundo, zone of inhibition, disc diffusion

## Introduction

Despite of the rich traditional Ayurvedic medical history of India but some herbs lacked adequate scientific documentation, particularly in light scientific knowledge.

*Vitex negundo* locally known as Nirkundi or Nallanocci. It is an aromatic large shrub or small tree about 3m in height with quadrangular branches and almost found throughout India <sup>[2, 3]</sup>. The previous study reveled that it plants like *Vitex negundo* like possesses antibacterial, antifungal activity and many pharmacological properties <sup>[9, 13-15]</sup>. The World Health Organization, report says that more than 80% of world's populations relied on traditional medicine for their primary health care needs <sup>[1]</sup>. The each part of this plant has a great medicinal importance but the leaves and the barks are the most widely used in the medicine field <sup>[4]</sup>.

The leaves extract work as tonic along with long pepper in catarrhal fever problem [4]. fresh leaves also plays vital role anti-inflammatory, analgesic and antihistamine activities [5]. Leave powder locally used as mosquito repellent. The root extract also considered as potent snake anti-venom [11].

So the present investigation was carries out to extract and screen for antibacterial activity of the *Vitex negundo* 

# **Materials and Methods**

## **Collection of plant material**

In the present work, *Vitex negundo* Linn leaves were collected from premises of Raje Dharamarao College of science, Aheri and used for phytochemical analysis and antimicrobial activity.

# Sample preparation

The *Vitex negundo* Linn leaves were collected and shade dried. Since certain compounds get denatured in sunlight, it is dried under shade to avoid decomposition. The dried leaves were then pulverized well in a mixer grinder and kept in an air tight container for further use.

#### **Preparation of Methanol extract**

The *Vitex negundo* Linn powder were extracted in methanol by using Soxhlet apparatus to determine antibacterial activity. 30 g of air-dried powder was taken and extracted with 300 ml methanol using Soxhlet apparatus. The extract was collected and dried. After that allowed for 10 cycles to complete and the apparatus was switched off and then the sample solution were taken and transferred in a beaker and covered it with a paper and holes was made on the paper for the evaporation of the solvent. Allowed it for drying and then the residue was collected from the beaker.

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#### **Bacterial pathogens**

The standard pathogenic bacterial cultures (Table 1) were procured from Guru Nanak College of Science, Ballarpur Chandrapur and used in the present study. The bacteria rejuvenated in Nutrient Agar. The inoculums size of the

bacteria culture was standardized according to the National Committee for Clinical Laboratory Standards (NCCLS, 2002) guidelines. The pathogenic bacterial culture was inoculated into sterile Nutrient agar and incubated at 37 °C for 3 hr. (Fig 3)

Table 1: List of pathogenic bacteria

1.	Escherichia coli
2.	Staphylococcus aureus
3.	Pseudomonas
4.	Enterobacter
5.	Salmonella typhimuri

#### **Inoculum preparation**

A total three bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* were procured from Pravara Medical Institute and Research Centre, Loni, Tal- Rahata and used in the present study. Bacterial inoculum was prepared by inoculating a loopful test organism in 5 ml nutrient broth and incubating at 37 °C for 3-4 hrs till moderate turbidity was developed.

# Preparation of growth media Nutrient agar

Nutrient agar (Peptone 5g, Beef extract 3g, Sodium chloride 8g, Agar 20g, pH 7.3±0.2, 1000 ml Distilled Water) was used in preparation of medium for growth of bacterial pathogens. Prepared nutrient agar was autoclaved at 121 °C and 15 lbs pressure, and then cooled the nutrient agar up to 45 °C and poured in sterile Petri plates under the aseptic conditions. After solidifying, kept for 24 hrs at room temperature for the checking of contamination in media, it is used for testing the antibacterial activity of herbal powder extracts.

#### Antimicrobial activity using disc diffusion method

The agar disc diffusion method was used for antibacterial assay. Petri plates were prepared by pouring 20 ml of Nutrient Agar mediumand allowed to solidify. Plates were solidified and 30µl of bacterial culture of *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* was poured and uniformly spread. The excess inoculum was drained away and the inoculum was allowed to dry for 5 minutes. Dried and sterilized filter paper disc (5 mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. The plates were incubated at 37° C for 24 hours. Antibacterial activity was evaluated by measuring the diameter of zones of inhibition against the tested bacteria (Fig 1 and Fig 2).

# Result and Discussion Antibacterial activity

In the present study, methanol extract was screened for antibacterial potential against enteric bacterial pathogen. They exhibited significant antibacterial activity against human pathogen *E. Coli, S. aureus* and *K. Pneumoniae*.

In the present investigation, the antibacterial activity of the leaf extract of *Vitex negundo* Linn assayed against three potentially pathogenic microorganism *E. Coli, S. aureus* and *K. Pneumonia* against same concentration of the extract to understand and the most effective activity.



**Fig 1:** Zone of inhibition in *E. coli* 

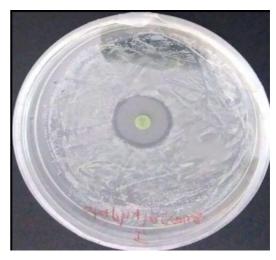


Fig 2: Zone of inhibition in Salmonella typhimuri

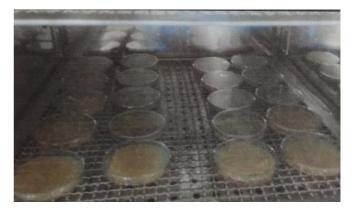


Fig 3: Plates for all 5 different human pathogens with four replications

**Table 2:** Zone of inhibition on different pathogens

	Concentration of leaf extract	Zone of inhibition			Maan
Solvent extract	(μ <b>l</b> )	Plate I (mm)	Plate II (mm)	Plate III (mm)	Mean
Escherichia coli	30	23.2	22.8	23.1	23
Staphylococcus aureus	30	22.1	21.8	22.4	22.1
Pseudomonas	30	20.2	19.3	19.4	19.6
Enterobacter	30	18.2	18.4	18.9	18.5
Salmonella typhimuri	30	17.6	17.4	17.8	17.8

The perusal of previous study indicates that the Ethyl acetate, ethanol and essential oil extracts of *V. negundo* Linn. has antibacterial activity <sup>[9]</sup>. Not only the leaves but also Crude ethanol extract of seeds also investigated for in vitro antifungal activity <sup>[10]</sup>. these is great scope for Screening of the plant under (*V. negundo*) with the help of MIC, MBC/MFC and TA determination to establish the extract as abtibiotic to utilize its antimicrobial potential.

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